# TECHNICAL MEMORANDUM

CH2MHILL

gdc

## BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT FEBRUARY 2000 SAMPLING

**Prepared For:** StarKist Samoa (NPDES Permit AS0000019)
COS Samoa Packing (NPDES Permit AS0000027)

**Prepared By:** Steve Costa/gdc
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**Date:** 18 April 2000

**Distribution:** Carl Goldstein
United States Environmental Protection Agency, Region 9
Sheila Wiegman
American Samoa Environmental Protection Agency

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### *Purpose*

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in February 1998. This is the 13th required semi-annual test. Separate technical memoranda have been being prepared to describe the results of concurrent effluent chemistry testing.

### *Study Objectives*

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES permits requires that semi-annual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the effluent.

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests. All comments from U.S. EPA have been incorporated into either the

Standard Operating Procedures or have been incorporated into the procedures used by the laboratory doing the test, Advanced Biological Testing, Inc., as documented in previous reports.

The bioassay tests were originally specified using the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests, a substitute species, *Mysidopsis bahia*, has been approved by U.S. EPA (CH2M HILL, 26 January 1995). For the February 2000 sampling, *Penaeus vannami* was not available and *Mysidopsis bahia* was used.

The effluent acute bioassay sampling must be concurrent with effluent sampling for chemical analysis. Effluent samples were collected as 24-hour composite samples. The effluent acute bioassay was conducted using a combined composite effluent sample made up from the composite effluent samples from the StarKist Samoa and COS Samoa Packing facilities, as approved by EPA. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

### *Effluent Sampling Methods*

Between 1200 on 10 February 2000 and 0900 on 11 February 2000, 24-hour, flow-weighted, composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites following the routine composite sample collection schedule for the plants. Detailed sampling procedures are described in the Standard Operating Procedures (SOP) which is provided in Attachment I.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24-hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times and the relative effluent volumes calculated from plant flow records are summarized in Table 1. The relative effluent volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was

completed and then sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL on flights from Pago Pago to Honolulu and then to San Francisco. The sample was received by the testing laboratory on 14 February 2000. The chain-of-custody form is provided in Attachment II.

### *Bioassay Testing Procedures*

The bioassay tests were conducted by Advanced Biological Testing Inc., Rohnert Park, California. The testing procedures and results of the bioassay tests are provided in the Laboratory report included as Attachment III. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA document EPA/600/4-90/027F, August 1993, as the source of methods for conducting the test.

The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports. A brine control was run and a comparison was made with the dilution water laboratory control. The test organisms were required to be 1 to 5 days old, with a 24-hour range in age, and the test temperature was to be held at 20 ±1°C or 25 ±1°C. For this bioassay, two-day-old *Mysidopsis bahia* were used.

Because of the demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD), discussed and documented in previous technical memoranda describing the first two bioassay tests, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, temperature, and ammonia. Additionally, a reference toxicant of sodium dodecyl sulfonate (SDS) was made up of a 2-gram per liter stock solution in distilled water and tested at concentrations of 25, 12.5, 6.25, 3.1, and 1.9 mg/L in 31 ppt seawater for a 96-hour test.

### *Results*

The results of the bioassay tests are summarized as follows:

> ***Mysidopsis bahia* Effluent Bioassay.** All results from the bioassay tests are included in Attachment III. The results of the mysid bioassay tests indicate

the LC50 for the effluent tested was 20 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 6.25 percent and the Least Observable Effects Concentration (LOEC) was 12.5 percent.

***Mysidopsis bahia* Reference Toxicant Bioassay.** The reference toxicant had a LC50 of 15.4 mg/l. The laboratory mean was 14.67±8.21 mg/l with the data falling within one standard deviation of the laboratory mean, indicating normal sensitivity.

### *Discussion*

Table 2 summarizes the results of the effluent bioassay tests for the samples collected in the February 2000 sampling compared to the previous bioassay tests. The LC50, NOEC and LOEC are within the range obtained from previous reports where *Mysidopsis bahia* was used in place of *Penaeus vannami*.

### *Conclusions*

The bioassay tests for the Joint Cannery Outfall effluent for February 2000 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 6.25% which was observed in February 2000, corresponds to a dilution of 16:1 which is achieved within a time frame of seconds and within a few meters of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

**Table 1**
**StarKist Samoa and COS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing**
**February 2000**

| Grab Sample Number | COS Samoa Packing | | StarKist Samoa | | COS Samoa Packing Percent of Total Flow | StarKist Samoa Percent of Total Flow |
|---|---|---|---|---|---|---|
| | Sampling Date and Time | Effluent Flow Rate (mgd) | Sampling Date and Time | Effluent Flow Rate (mgd) | | |
| 1 | 10 Feb 2000 1200 | 0.60 | 10 Feb 2000 1200 | 1.59 | 4 | 9 |
| 2 | 1500 | 0.64 | 1500 | 1.24 | 4 | 7 |
| 3 | 1800 | 0.64 | 1800 | 0.98 | 4 | 6 |
| 4 | 2100 | 0.52 | 2100 | 1.48 | 3 | 9 |
| 5 | 2400 | 0.52 | 2400 | 1.71 | 3 | 10 |
| 6 | 11 Feb 2000 0300 | 0.52 | 11 Feb 2000 0300 | 1.77 | 3 | 11 |
| 7 | 0600 | 0.52 | 0600 | 1.66 | 3 | 10 |
| 8 | 0900 | 0.66 | 0900 | 1.73 | 4 | 10 |
| **Total** | | 4.62 | | 12.16 | 28 | 72 |
| **Mean** | | 0.58 | | 1.52 | | |

**Table 2**
**StarKist Samoa and COS Samoa Packing**
**Combined Effluent Bioassay Results**

| Date | Species | Parameters | | |
|---|---|---|---|---|
| | | LC 50 | NOEC | LOEC |
| 2/93 | *Penaeus vannami* | 4.8%[1] | 3.1% | 6.25% |
| 10/93 | *Penaeus vannami* | 15.67% | 3.1% | 6.25% |
| 2/94 | *Penaeus vannami* | 15.76% | <1.6% | 1.6% |
| **10/94** | ***Mysidopsis bahia***[2] | **31.2%** | **25%** | **50%** |
| 3/95 | *Penaeus vannami* | 14.8% | 6.25% | 12.5% |
| **3/95** | ***Mysidopsis bahia***[3] | **10.8%** | **6.25%** | **12.5%** |
| 2/96 | *Penaeus vannami* | >50% | >50% | >50% |
| **2/96** | ***Mysidopsis bahia***[3] | **28.36%** | **12.5%** | **25%** |
| 3/96 | *Penaeus vannami* | 44.4% | 25% | 50% |
| 11/96 | *Penaeus vannami* | 7.11% | 3.1% | 6.25% |
| 03/97 | *Penaeus vannami* | 39.36% | 12.5% | 25% |
| 09/97 | *Penaeus vannami*[4] | 12.3% | 6.25% | 12.5% |
| **06/98** | ***Mysidopsis bahia***[2] | **17.2%** | **6.25%** | **12.5%** |
| **11/98** | ***Mysidopsis bahia***[2] | **15%** | **6.25%** | **12.5%** |
| **02/00** | ***Mysidopsis bahia***[2] | **20%** | **6.25%** | **12.5%** |

[1]The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

[2]*Mysidopsis bahia* substitutes as *Penaeus vannami* not available, as directed by U. S. EPA.

[3]*Mysidopsis bahia* used in addition to *Penaeus vannami* as described in text. Only one species is required by the permit conditions.

[4]Stage 1 (3 mm) *Penaeus vannami* were used for testing as older Stage 7 and 8 (8-10 mm) *Penaeus vannami* were not available.

# ATTACHMENT I

## EFFLUENT SAMPLING STANDARD OPERATING PROCEDURES

**Joint Cannery Outfall**
**Effluent Sampling for Chemistry and Bioassay**
**Toxicity Testing**

**Revision 4**
**26 January 2000**

# Standard Operating Procedures
# Joint Cannery Outfall (JCO)
# Effluent Sampling for Chemistry
# and Bioassay Toxicity Testing

## Introduction

StarKist Samoa and COS Samoa Packing are required by their NPDES permits to conduct semiannual priority pollutant analyses (effluent chemistry) and definitive acute bioassays on their cannery wastewater effluent. The following gives detailed procedures for collecting, preparing, and shipping effluent samples for these analyses. The effluent chemistry and bioassay analyses are to be conducted on simultaneous samples. Therefore, this standard operating procedure (SOP) addresses collection of samples for both tests as a single set of procedures. The chemical analyses are done on each cannery's wastewater effluent separately and the bioassay test is done on a combined composite from each cannery.

## Overview

The following cannery wastewater effluent samples must be collected and prepared for shipment to the appropriate laboratory:

- Composite samples of cannery effluent from the StarKist Samoa, facility for chemical analysis
- Composite samples of cannery effluent from the COS Samoa Packing facility for chemical analysis
- A composite sample of combined effluent from both StarKist Samoa and COS Samoa Packing for acute bioassay tests

Each of the effluent chemistry samples will be a composite of eight (8) grab samples taken over a 24-hour period. The bioassay sample will be a composite of sixteen (16) grab samples, eight (8) from StarKist Samoa and eight (8) from COS Samoa Packing, collected over the same 24 hour period. A schematic flowchart of the sample compositing and splitting for the various analyses are shown on Figure 1.

Sampling requires a coordinated effort by both canneries. The canneries should conduct their sampling so that samples are collected on approximately the same schedules. If possible, the sampling should be scheduled so that the samples are composited the day they are shipped to the laboratories for analysis. An example sampling and shipping schedule is shown in Table 1.

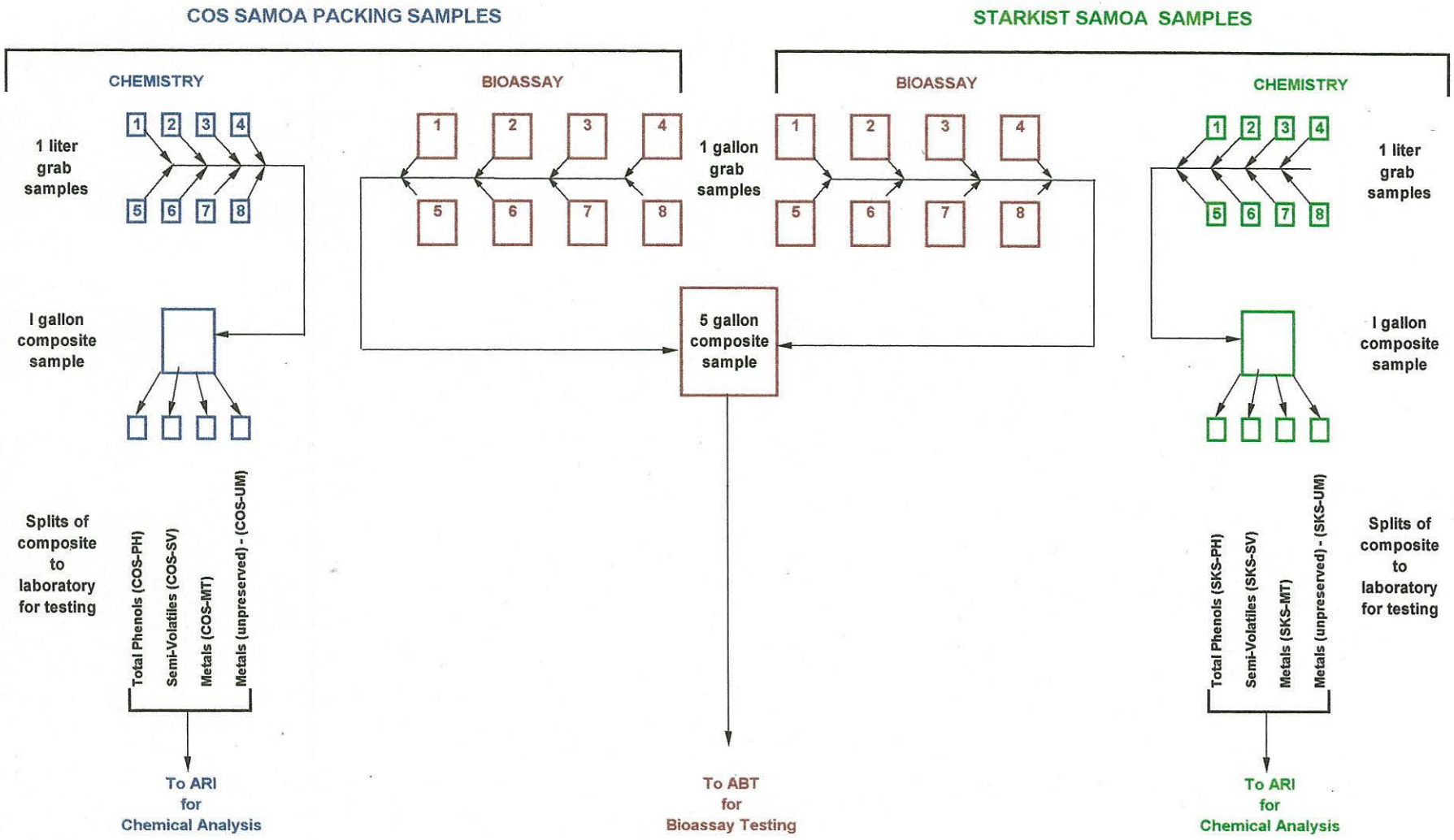


**Figure 1.**
**Schematic Flow Diagram of Joint Cannery Outfall Wastewater Effluent Sampling for Chemical Toxicity**

| Table 1<br>Example Schedule for Sample Collection and Shipping | |
|---|---|
| **Time** | **Activity** |
| Thursday 12:00 noon through<br>Friday 9:00 AM | Collect grab samples from both canneries for chemistry and bioassay tests |
| Friday<br>9:00 AM - 12:00 noon | Composite samples in cannery laboratory |
| Friday<br>1:00 PM - 3:00 PM | Prepare samples for shipping |
| Friday<br>4:00 PM | Deliver coolers containing samples to DHL or Federal Express |

The above example schedule assumes samples are shipped on a Friday evening flight (note that flight schedules can change and the sampling should be scheduled to minimize holding times). The above schedule shall be modified based on the availability of laboratory personnel and airline schedules, however, the samples should be composited on the day of the scheduled flight and sampling should take place during the 24 hours just before compositing the samples. The only exceptions are: [1] possible weekend shipments where samples should always be collected after 12 noon on Monday and before 12 noon on Friday, [2] scheduled changes or shutdown in plant operations, in which case samples should be collected during normal plant operations as closely as possible to available shipping time.

## List of Equipment/Supplies

Lists of the supplies that should be on hand or readily available that will be required for collecting, compositing, and shipping the effluent samples are given below. (Note: items marked with an asterisk (*) will be supplied by CH2M HILL or by the laboratory performing the analyses).

## *Sample Collection (required per facility)*

For sample collection the following supplies are required:

- * Eight (8) 1-liter amber glass sampling bottles (plus 8 backup bottles)
- * Eight (8) 1-gallon cubitainers or other appropriate containers (plus 2 backup cibitainers)
- * Labels and permanent marker for marking sample containers
- * Gloves
- Ice chests with ice (or refrigerator space) for storing samples (There should be sufficient storage space for storing all containers listed above)
- * Worksheet for recording time and flow (provided in Attachment A and separately in chemistry supply cooler shipped to canneries)

## *Compositing and Preserving Samples*

For sample compositing and preservation the following are needed:

- * Two (2) Chemistry bottle kits (one for each cannery) (cooler + containers, see contents listed in Table 2)
- * Two (2) 1-gallon glass bottle for chemistry composite (one for each cannery)
- * 5-gallon cubitanier for bioassay composite
- * Clean graduated cylinder(s) for compositing effluent samples:
  1000 ml cylinder for bioassay composites
  250 ml cylinders for chemistry composites
- * $H_2SO_4$ for preserving total phenol samples
- * $HNO_3$ for preserving metals samples
- * pH test paper for checking pH of preserved samples
- * Labels and permanent marker for identifying samples
- Cubed ice (as required)
- * Compositing worksheets (Attachments A and B)
- Calculator

## *Shipping samples*

For sample packaging and shipping the supplies required include:

- * One cooler for bioassay composite
- * Two coolers for prepared chemistry samples (one for each cannery)
- Cubed ice (as needed)
- * Large or Extra Large zip-lock/freezer bags
- * Chain-of-Custody Forms for each cooler (Actual ones filled out are included in the Chemistry supply folder)
- * Shipping labels
- * Strapping Tape and clear shipping tape
- DHL /or FedEx airbills
- * Return manifests (examples included in chemistry folder)

**Table 2**
**Contents of Effluent Chemistry Bottle Kits**
**(one for each cannery)**

| Sample Container | Qty | Chemical Parameter | Sample Preservative |
|---|---|---|---|
| 1-liter amber glass | 1 | Semivolatile Organics | none |
| 500 ml plastic | 1 | Total Phenols | $H_2SO_4$ |
| 500 ml plastic | 1 | Inorganics/Metals[1] | $HNO_3$ |
| 500 ml plastic | 1 | Inorganics/Metals[1,2] | NONE[2] |

[1] Metals include: Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Selenium, Silver, and Zinc.

[2] **There are two samples for metals**: one preserved and one not preserved (will be used with coprecipitation for certain metals if detection limits cannot be achieved).

## Sampling

Eight pairs of samples will be collected at each cannery over a 24-hour period. The samples should be collected from normal accepted sampling locations at which the flow rate is known. Samples shall be collected at intervals of approximately three hours. The general procedure for collecting samples is outlined below:

1) Label a 1-liter sampling jar and a 1-gallon container with sample number to be collected, time and date of sample collection, and flow rate during sampling. Labeling should be done with a permanent marker on a waterproof label. Plastic containers may be written on directly.

   The convention used for labeling samples should identify the facility in the first part of the label (SKS = StarKist Samoa, COS = COS Samoa Packing) and the grab sample sequence number (e.g. COS-2, for the second sample in the series). Write down the date, time, and flow rate on the appropriate row in columns A, B, and C of the Worksheet for Compositing Effluent Chemistry Samples (Attachment A).

2) **Chemistry Sample.** Rinse a 1-liter sampling jar with effluent. Fill jar to the top and cover securely with its lid. If samples are collected from a tap in the line, fill the sample container directly from the tap. If samples are collected from a flume requiring the container to be dipped under the surface, use a separate container to remove the effluent from the flume and fill the sample

container. Any container used for sampling should be clean and rinsed with effluent prior to collecting each sample.

3) **Bioassay Sample.** Collect the bioassay sample in the 1 gallon sampling container in the same manner as the chemistry sample. Rinse the 1 gallon container, and any other sampling container used, with effluent prior to filling.

4) Store all samples in coolers on ice or refrigerator at a temperature of approximately 4 °C. Do **NOT** store samples in a freezer or by using a method that would freeze the sample.

## Sample Preparation/Compositing

The samples will be composited in the StarKist Samoa and/or COS Samoa Packing laboratories. The effluent chemistry samples from each cannery can be composited and prepared separately in each facility's lab. The bioassay samples must be composited together and all bioassay samples will need to be delivered to one lab or the other. The area used for compositing should include a sink and clear tabletop area that is clean and dry. Basic steps used to composite the effluent chemistry and bioassay samples are listed below. Worksheets for calculating composite volumes are included as Attachments A and B for the chemistry and bioassay samples, respectively. Example completed forms, including worksheets for chemical and bioassay composite sample preparation and chain-of-custody forms are included as Attachment C.

### *Effluent Chemistry*

1) **Label Containers.** If this has not already been done by the laboratory, the containers listed in Table should be labeled and placed on the table. The labels will be used to identify the samples by the lab and should be descriptive of the samples. These codes will also be used on the Chain-of-Custody forms that will be attached to each cooler. An example Chain-of-Custody form is included in Attachment C. The convention used for these samples identifies the facility in the first part of the label (SKS = StarKist Samoa, COS = COS Samoa Packing) and the type of analysis in the second (MT = metals (preserved sample), UM = metals (unpreserved sample), SV = semi-volatiles, PH= phenols. This or a similar convention shall be used for labeling the samples. Labels should also indicate whether and what kind of preservative is used.

2) **Calculate Composite Volumes.** The worksheet included as Attachment A should be used to calculate volumes of each of the eight individual samples that will be required to be composited into a single sample from which subsamples will be prepared for laboratory analysis[1]. Columns A through C should be filled out during the sample collection. Instructions for filling out the remainder of the table are included on the worksheet. Column D in the worksheet represents the fraction of the composited sample that should come from the individual sample represented by that row. Columns E, F, and G give the volume of each individual sample that is required to produce 1-gallon, 1-liter and 500-ml samples, respectively. The bottom row of the table, labeled "Totals:", are totals from the columns above them. The box labeled TF is used to calculate the numbers in column D. The other boxes are used to check arithmetic and should be equal to the numbers in parenthesis below them. **These forms must be retained and copies sent or faxed to CH2M HILL for use in preparing the report to EPA.** Unless notified otherwise send the forms via U.S. mail (use P.O. Box) or Fax to:

Karen Glatzel
216 Driftwood Lane
P.O. BOX 1238
Trinidad, CA 95570-1238
Phone: 707-677-0123
Fax: 707-677-9210

3) **Composite and Preserve Samples.** Volumes calculated in Column E of the worksheet should be used to composite samples into a 1 gallon glass bottle.[1] A clean graduated cylinder should be used to measure the effluent. Prior to compositing the samples, the cylinder should be rinsed with a dilute solution of nitric acid ($HNO_3$), rinsed out with de-ionized or distilled water, and finally rinsed with effluent. (Precleaned cylinders may be supplied with the sample bottles.)

Chemistry sample containers listed in Table should be filled from the composite sample. **Samples requiring preservatives should be treated at this time.** For total phenols the preservative is sulfuric acid and for the preserved metals sample the preservative is nitric acid. The samples will probably require 5 ml of acid (as supplied in 5 ml ampoules) or more. The pH of the phenols and preserved metals samples should be checked and adjusted to be less 2.[2]

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[1] Alternatively, volumes calculated in Column F can be used for 1000 and 500 ml composite samples.

[2] Alternatively, sample containers to be shipped to the laboratory for chemical analysis may have been prepared in the laboratory and the approximate amount and correct type of preservative may already be in each bottle. However, the pH will still need to be checked and additional preservative may be necessary.

Securely tighten the caps and shake the sample container well prior to testing pH. To check the pH it is recommended to pour a small amount of preserved sample into a small container such as an extra bottle cap. Dip a pH strip into this sample and check for a pH less than 2. If the pH is above 2, continue to add a few drops of the appropriate acid to the sample, recap and shake, and repeat the checking procedure. Check sample container caps following preservation to make sure the container lids are securely tightened.

3) **Complete Chain-of-Custody Form(s).** At least one chain-of-custody form is required for each cooler of samples that will be shipped. An example of a completed Chain-of-Custody form is included as part of Attachment C. Sample identification on the Chain-of-Custody should match the labels on the sample containers exactly. COS Samoa Packing and StarKist Samoa effluent chemistry samples should be shipped in separate coolers. Blank or partially filled out chain of custody forms may be provided with the sample bottles.

**One copy of each Chain-of-Custody should be retained at the cannery and sent to Karen Glatzel** as described above for the compositing worksheet.

5) **Package Samples for Shipping.** Each sample jar should be wrapped in bubble-wrap or an equivalent packaging material and placed in a plastic zip-lock bag. Glass containers should be wrapped in two layers of bubble-wrap at a minimum. As much air as possible should be removed from the bag prior to sealing it. Too much air inside the bags will expand during the flight and pop the bag open. All chemistry samples from one cannery should be packaged in a single cooler if possible. Place sample jars inside the cooler. Packaging material (bubble wrap or equivalent) should be placed in the cooler to prevent containers from moving and impacting each other.

Ice or an equivalent means (such as chemical cold packs) must be included to keep the samples cold during shipping. Do not use dry ice to pack the samples. If ice is used, precautions should be taken to prevent melted ice from leaking out of the cooler during shipping. These include taping any drain plugs in the cooler shut with duct tape or strapping tape, and "double-bagging" the ice cubes in zip-lock bags, i.e. sealing the ice cubes in one bag, then sealing the bag containing ice in a second bag. As with the bags used to hold the sample jars, as much air as possible should be removed from the bags prior to sealing.

The Chain-of-Custody form for each cooler should be signed, placed in a zip-lock bag, and taped to the inside of the cooler lid. The cooler should be taped securely shut with strapping tape or other strong packaging tape to prevent it from opening during shipping. Mailing labels should be placed inside (with the chain-of-custody forms) and outside (in addition to the airbill).

6) **Shipping.** Ship the chemistry samples by express freight (DHL or Federal Express) to the laboratory as directed for each sampling period. For the February 2000 sampling ship the chemistry samples to:

Ms. Jennifer Baier
Analytical Resources Incorporated
333 Ninth Avenue North
Seattle, WA 98109-5187
Phone: (206) 621-6490
FAX: (206) 621-7523

Or to the person and laboratory as directed by the project manager, if different from above.

## *Effluent Bioassay*

1) Calculate Composite Volumes. The worksheet included as Attachment B should be used to calculate volumes of each of the 16 individual samples that will be required to be composited into the 2 1/2-gallon cubitainer. Columns A and B should be filled out based on the flows recorded during sampling onto the effluent chemistry compositing worksheet.

Note that flows must be recorded in million gallons per day (mgd). If flows are greater than about 2, they are probably recorded in gallons per minute (gpm). If flows are reported in gpm they should be converted by multiplying the recorded flow by 0.0014. Instructions for filling out the remainder of the table are included on the worksheet. Columns C and D in the worksheet represent the fraction of the composited sample that should come from individual samples taken at each cannery. Columns E and F give the volume of each individual sample that is required to produce a 2 1/2-gallon composited sample, the individual volumes must be adjusted for an alternative volume (For example, for a five-gallon sample double the amounts shown in columns E and F.). The bottom row of the table, labeled "Totals:", are totals from the columns above them. The box labeled TF is used to calculate the numbers in columns C and D. The other boxes are used to check arithmetic and should be equal to the numbers in parenthesis below them. **This forms must be retained and copies sent via U.S. Mail (use P.O. Box) or Faxed to Karen Glatzel for use in preparing the report to EPA.** Unless notified otherwise send the forms to:

Karen Glatzel
216 Driftwood Lane
P.O. BOX 1238
Trinidad, CA 95570-1238
Phone: 707-677-0123
Fax: 707-677-9210

**On special instruction from the laboratory the volume required may change. Check with the project manager prior to initiating sampling, since the volume required for individual grab samples will need to be modified if the required size of the composite sample is increased.**

3) **Composite Samples.** Volumes calculated in Columns E and F of the worksheet should be used to create 2 1/2-gallon (or alternative volume) composite sample. A clean graduated cylinder should be used to measure the effluent. Prior to compositing the samples, the cylinder should be rinsed with a dilute solution of nitric acid ($HNO_3$), rinsed out with de-ionized or distilled water, and finally rinsed with effluent from one of the samples. (Precleaned cylinders may be supplied.)

The cubitainer holding the sample should be clearly marked as "JCO Effluent Bioassay Sample." The graduated cylinder should be used to fill the cubitainer with the appropriate volumes from each of the 16 sample containers. Excess air should be squeezed out of the container prior to capping it.

4) **Complete Chain-of-Custody Form.** Chain-of-Custody forms will be included with the effluent chemistry sample containers. One Chain-of-Custody form is required the composite bioassay sample. An example of a completed chain-of-custody form is included as part of Attachment C. **One copy of each chain of custody should be retained at the cannery and sent to Steve Costa** as described above for the compositing worksheet.

5) **Package Sample for Shipping.** The cubitainer holding the bioassay sample should be placed in a separate cooler. The bioassay and effluent chemistry samples described above will be sent to separate labs. Therefore, these should not be packaged together. Ice or an equivalent means (such as chemical cold packs) should be used to fill in the empty space in the cooler and keep the samples cold during shipping. If ice is used, precautions should be taken to prevent the melted ice from leaking out of the cooler during shipping. These include taping any drain plugs in the cooler shut with duct tape or strapping tape, and "double-bagging" the ice cubes in zip-lock bags, i.e. sealing the ice cubes in one bag, then sealing the bag containing ice in a second bag. As described for the effluent chemistry samples described above, as much air as possible should be removed from the bags prior to sealing.

The chain-of-custody form should signed, placed in a zip-lock bag, and taped with duct tape to the inside of the cooler lid. The cooler should be taped securely with strapping tape or other strong packaging tape to prevent it from opening during shipping. Mailing labels should be placed inside (with the chain-of-custody forms) and outside (in addition to the airbill).

6) **Shipping.** Ship the bioassay sample to the laboratory as directed for each sampling. For the February 2000 sampling ship the bioassay sample to:

Dr. Kurt Kline
Advanced Biological Testing, Inc.
5685 Redwood Drive, Suite 105
Rohnert Park, CA 94928
(707) 588-2880 phone
(707) 588-2884 Fax

## Health and Safety Considerations

The sample collection and compositing should be done or directly supervised by staff that are experienced with this type of work and are fully aware of all health and safety practices that apply in such cases. The canneries will require that all staff in the facility wear long pants and closed shoes (no sandals). In addition cannery personnel will brief project staff on evacuation routes and other safety issues as required. Head and hearing protection must be available and worn in designated areas while in the canneries and cannery personnel will provide project staff with hats and earplugs. While collecting samples from the effluent flumes, gloves and appropriate eye protection must be worn. Floors, decks, and ladders are often slippery and shoes with appropriate sole material should be selected for work in the canneries.

The above description is not intended to be comprehensive or exhaustive. Always work with experienced staff and when in doubt ask cannery personnel about correct policies and procedures.

## Attachment A
## Worksheet for Compositing Effluent Chemical Samples

**Send completed worksheets to: Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570**

**Facility:** **Date:**

| Worksheet for Compositing Effluent Chemistry Samples | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Sample Collection Time | | (C) Flow | (D) Fraction of Total Flow | Volume of sample (note: 1 gallon = 3780 ml) | | |
| | (A) Date | (B) Time | | | (E) 1 gallon | (F) 1 liter | (G) 500 ml |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
| | | Totals: | | | | | |
| | | | (TF) | (1.0) | 3780 | 1000 | 500 |

Instructions:

1) Fill in date and time each sample was taken (columns A and B) and recorded flow rate at the time of the sample (column C).

2) Add all flows and record in box below column C (TF)

3) Calculate fraction of total flow (column D) for each flow rate in column C:

Fraction of total (D) = Flow (C) ÷ Total flow (TF)

4) Calculate volume of collected sample for 1-gallon, 1-liter, or 500 ml chemistry sample containers (columns E and F):
(E) = (D) x 3780
(F) = (D) x 1000
(G) = (D) x 500

5) Check calculations. Sum columns D, E, F, and/or G and record totals in boxes below each column. Numbers should match numbers in parenthesis below the boxes.

## Attachment B
## Worksheet for Compositing Effluent Bioassay Samples

**Send completed worksheets to: Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570**

Facility: Date:

| Worksheet for Compositing Effluent Bioassay Samples | | | | | | |
|---|---|---|---|---|---|---|
| No. | (A) COS Flow (mgd) | (B) SKS Flow (mgd) | Fraction of Total Flow | | Volume of sample for 2 1/2 gal container (ml) (Note: 2 ½ gallons = 9500 ml) | |
| | | | (C) COS (A)÷(TF) | (D) SKS (B)÷(TF) | (E) COS (C)x9500 | (F) SKS (D)x9500 |
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| Totals: | | | | | | |
| | Total (A)+(B): | | Total (C)+(D): | | Total (E)+(F): | |
| | | (TF) | | (1.0) | | (9500 ml) |

**Instructions:**

1) Fill in recorded flow rates in columns A and B. Flow rates should be in mgd. (Note: StarKist flowrates may be in gpm. If flows are greater than about 2, they are probably measured as gpm. To convert, multiply the flow recorded in gpm by 0.0014).

2) Total flows for COS and SKS in columns A and B. Sum the totals and write total in box labeled (TF).

3) Calculate fraction of total flow for each sample (columns C and D). To check calculations, total columns C and D and add totals together in box at bottom of column D. Total should equal 1.0

4) Calculate volume required of each collected sample to fill a 2 1/2-gallon container (columns E and F):
   (E) = (C) x 9500
   (F) = (D) x 9500
   **For an alternative volume the constant must be adjusted, for example for a 5-gallon container:**
   (E) = (C) x 19000
   (F) = (D) x 19000

5) Check by totaling columns E and F. Sum of E + F should equal approximately 9500 for a 2 1/2 gallon container.

## Attachment C
## Example Worksheets and Chain-of-Custody Forms

**Send completed worksheets to: Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570**

Facility: **COS Samoa Packing** Date: 2/10/00

| Worksheet for Compositing Effluent Chemistry Samples | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Sample Collection Time | | (C) Flow | (D) Fraction of Total Flow | Volume of sample (note: 1 gallon = 3780 ml) | | |
| | (A) Date | (B) Time | **mgd** | | (E) 1 gallon | (F) 1 liter | (G) 500 ml |
| 1 | 2/10/00 | 1000 | 0.26 | 0.06 | 227 | 60 | 30 |
| 2 | | 1300 | 0.60 | 0.12 | 454 | 120 | 60 |
| 3 | | 1600 | 0.64 | 0.13 | 491 | 130 | 65 |
| 4 | | 1900 | 0.64 | 0.13 | 491 | 130 | 65 |
| 5 | | 2200 | 0.64 | 0.13 | 491 | 130 | 65 |
| 6 | 2/11/00 | 0100 | 0.68 | 0.14 | 529 | 140 | 70 |
| 7 | | 0400 | 0.68 | 0.14 | 529 | 140 | 70 |
| 8 | | 0700 | 0.72 | 0.15 | 567 | 150 | 75 |
| | | Totals: | 4.86 | 1.000 | 3779 | 1000 | 500 |
| | | | (TF) | (1.0) | (3780) | (1000) | (500) |

**Instructions:**

1) Fill in date and time each sample was taken (columns A and B) and recorded flow rate at the time of the sample (column C).

2) Add all flows and record in box below column C (TF)

3) Calculate fraction of total flow (column D) for each flow rate in column C:

Fraction of total (D) = Flow (C) ÷ Total flow (TF)

4) Calculate volume of collected sample for 1-gallon, 1-liter, or 500 ml chemistry sample containers (columns E and F):
(E) = (D) x 3780
(F) = (D) x 1000
(G) = (D) x 500

5) Check calculations. Sum columns D, E, and F and record totals in boxes below each column. Numbers should match numbers in parenthesis below the boxes.

**Send completed worksheets to: Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570**

Facility: **StarKist Samoa** Date: 2/10/00

| Worksheet for Compositing Effluent Chemistry Samples | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Sample Collection Time | | (C) Flow | (D) Fraction of Total Flow | Volume of sample (note: 1 gallon = 3780 ml) | | |
| | (A) Date | (B) Time | **gpm** | | (E) 1 gallon | (F) 1 liter | (G) 500 ml |
| 1 | 2/10/00 | 1000 | 1025 | 0.154 | 582 | 154 | 77 |
| 2 | | 1300 | 800 | 0.120 | 454 | 120 | 60 |
| 3 | | 1600 | 900 | 0.135 | 510 | 135 | 68 |
| 4 | | 1900 | 775 | 0.116 | 438 | 116 | 58 |
| 5 | | 2200 | 800 | 0.120 | 454 | 120 | 60 |
| 6 | 2/11/00 | 0100 | 775 | 0.116 | 438 | 116 | 58 |
| 7 | | 0400 | 850 | 0.127 | 480 | 127 | 63 |
| 8 | | 0700 | 750 | 0.112 | 423 | 112 | 56 |
| | | Totals: | 6675 | 1.000 | 3779 | 1000 | 500 |
| | | | (TF) | (1.0) | (3780) | (1000) | (500) |

**Instructions:**

1) Fill in date and time each sample was taken (columns A and B) and recorded flow rate at the time of the sample (column C).

2) Add all flows and record in box below column C (TF)

3) Calculate fraction of total flow (column D) for each flow rate in column C:

Fraction of total (D) = Flow (C) ÷ Total flow (TF)

4) Calculate volume of collected sample for 1-gallon, 1-liter, or 500 ml chemistry sample containers (columns E and F):
(E) = (D) x 3780
(F) = (D) x 1000
(G) = (D) x 500

5) Check calculations. Sum columns D, E, and F and record totals in boxes below each column. Numbers should match numbers in parenthesis below the boxes.

**Send completed worksheets to: Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570**

Facility: **JCO, StarKist and COS Samoa Packing** Date: 2/11/00

| Worksheet for Compositing Effluent Bioassay Samples | | | | | | |
|---|---|---|---|---|---|---|
| No. | (A) COS Flow (mgd) | (B) SKS Flow (mgd) | Fraction of Total Flow | | Volume of sample for 2 ½-gal container (ml) | |
| | | | (C) COS (A)÷(TF) | (D) SKS (B)÷(TF) | (E) COS (C)x9500 | (F) SKS (D)x9500 |
| 1 | 0.26 | 1.48 | 0.018 | 0.102 | 171 | 969 |
| 2 | 0.60 | 1.15 | 0.041 | 0.079 | 390 | 751 |
| 3 | 0.64 | 1.3 | 0.044 | 0.090 | 418 | 855 |
| 4 | 0.64 | 1.12 | 0.044 | 0.077 | 418 | 732 |
| 5 | 0.64 | 1.15 | 0.044 | 0.079 | 418 | 751 |
| 6 | 0.68 | 1.12 | 0.047 | 0.077 | 447 | 732 |
| 7 | 0.68 | 1.23 | 0.047 | 0.085 | 447 | 808 |
| 8 | 0.72 | 1.08 | 0.050 | 0.075 | 475 | 713 |
| Totals: | 4.86 | 9.63 | 0.335 | 0.664 | 3184 | 6311 |
| | Total (A)+(B): | 14.49 | Total (C)+(D): | 0.999 | Total (E)+(F): | 9495 |
| | | (TF) | | (1.0) | | (9500 ml) |

**Instructions:**

1) Fill in recorded flow rates in columns A and B. Flow rates should be in mgd. (Note: StarKist flowrates may be in gpm. If flows are greater than 10, they are probably measured as gpm. To convert, multiply the flow recorded in gpm by 0.00144).

2) Total flows for COS and SKS in columns A and B. Sum the totals and write total in box labeled (TF).

3) Calculate fraction of total flow for each sample (columns C and D). To check calculations, total columns C and D and add totals together in box at bottom of column D. Total should equal 1.0

4) Calculate volume required of each collected sample to fill a 2 1/2-gallon container (columns E and F):

   (E) = (C) x 9500
   (F) = (D) x 9500

   See Attachment B for the adjustment required for a different size container.

5) Check by totaling columns E and F. Sum of E + F should equal approximately 9500 for a 2 1/2 gallon container.

**Example Chain-of-Custody for COS Samoa Packing Chemistry Samples and StarKist Samoa Chemistry Samples.**

**Send Cannery's copies of Chain-of-Custody forms to:**

**Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570**

**CH2MHILL Analytical Services**
*CHAIN OF CUSTODY RECORD*
*AND AGREEMENT TO PERFORM SERVICES*


☐ LMG 2567 Fairlane Drive, Montgomery, AL 36116-1622, (334) 271-1444 FAX (334) 271-3428
☐ LRD 5090 Caterpillar Road, Redding, CA 96003-1412, (916) 244-5227 FAX (916) 244-4109
☐ LKW Canviro Analytical Laboratories, Inc., 50 Bathurst, Unit 12, Waterloo, Ontario, Canada N2V 2C5, (519) 747-2575 FAX (519) 747-3806
☐ CVO 2300 NW Walnut Boulevard, Corvallis, OR 97330-3638, (541) 752-4271 FAX (541) 752-0276


COC # _____

| | |
|---|---|
| Project # 147323.JC.BA | Purchase Order # |
| Project Name: COS CHEMISTRY SCAN | |
| Company Name: CH2M HILL/gdc | |
| Project Manager or Contact & Phone #: KAREN GLATZEL, STEVE COSTA | Report Copy to: SAME, 707-677-0123 |
| Requested Completion Date: | Site ID |
| Sample Disposal: Dispose ☐ Return ☐ | |

| Sampling Date | Sampling Time | Type COMP | Type GRAB | Matrix WATER | Matrix SOIL | Matrix AIR | CLIENT SAMPLE ID (9 CHARACTERS) | LAB QC | TOTAL # OF CONTAINERS | Preservative: $H_2SO_4$ | — | NONE | $HNO_3$ | $HNO_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | X | | X | | | COS-PH | | 1 | X | | | | |
| | | X | | X | | | COS-SV | | 1 | | X | | | |
| | | X | | X | | | COS-MT | | 1 | | | | X | X |
| | | X | | X | | | COS-UM | | 1 | | | X | | |

DL (µg/L) · Requested Analytical Method #

| PHENOLS 420.1 @ DL=10 | SEMIVOLATILES 625 @ 10-100 | COPPER USING COPRECIPITATION | As-228.2-DL=5 | Cd-200.7-DL=5 | Cr-200.7-DL=10 | Co-220.2-DL=2 | Pb-239.2-DL=5 | Hg-245.1-DL=0.4 | Se-270.1-DL=5 | Ag-272.2-DL=2 | Zn-200.7-DL=20 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | X | X | X | X | X | X | X | X | X |

THIS AREA FOR LAB USE ONLY

| | |
|---|---|
| Lab # | Page / of |
| Lab PM | Custody Review |
| Log In | LIMS Verification |
| pH | Custody Seals Y N |
| | Ice Y N |
| QC Level 1 2 3 Other | |
| Cooler Temperature | |

| Alternate Description | Lab ID |
|---|---|
| | |

| | | | |
|---|---|---|---|
| Relinquished By / Empty Bottles | | Date/Time | Received By / Empty Bottles | Date/Time |
| Sampled By and Title (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) | Date/Time |
| Received By (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) | Date/Time |
| Received By (Please sign and print name) | | Date/Time | Shipped Via: UPS Fed-Ex Other _____ | Shipping # |

Special Instructions: SAVE SAMPLE FOR 30 DAYS AFTER REPORTING

26 Jan 2000

22

JCO:SOP:REV4

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*AND AGREEMENT TO PERFORM SERVICES*

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☐ LRD 5090 Caterpillar Road, Redding, CA 96003-1412, (916) 244-5227 FAX (916) 244-4109
☐ LKW Canviro Analytical Laboratories, Inc., 50 Bathurst, Unit 12, Waterloo, Ontario, Canada N2V 2C5, (519) 747-2575 FAX (519) 747-3806
☐ CVO 2300 NW Walnut Boulevard, Corvallis, OR 97330-3638, (541) 752-4271 FAX (541) 752-0276

COC # _____

| | |
|---|---|
| Project # 147323.JC.BA | Purchase Order # |
| Project Name: SKS CHEMISTRY SCAN | |
| Company Name: CH2M HILL/gdc | |
| Project Manager or Contact & Phone #: KAREN GLATZEL, STEVE COSTA | Report Copy to: SAME, 707-677-0123 |
| Requested Completion Date: | Site ID |
| Sample Disposal: ☐ Dispose ☐ Return | |

| Sampling Date | Sampling Time | Type: COMP | Type: GRAB | Matrix: WATER | Matrix: SOIL | Matrix: AIR | CLIENT SAMPLE ID (9 CHARACTERS) | LAB QC | TOTAL # OF CONTAINERS | Preservative: $H_2SO_4$ | Preservative: — | Preservative: NONE | Preservative: HNO3 | Preservative: H NO3 | Requested Analytical Method # (DL ug/L): PHENOLS 420.1 @ DL=10 | SEMIVOLATILES 625 @ 10-100 | COPPER USING COPPER PYRITHIONE PRECIPITATION | As - 206.2 - DL=5 | Cd - 200.7 - DL=5 | Cr - 200.7 - DL=10 | Cu - 220.2 - DL=2 | Pb - 239.2 - DL=5 | Hg - 245.1 - DL=0.4 | Se - 270.1 - DL=5 | Ag - 272.2 - DL=2 | Zn - 200.7 - DL=20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | X | | X | | | SKS-PH | | 1 | X | | | | | | | | | | | | | | | |
| | | X | | X | | | SKS-SV | | 1 | | X | | | | | | | | | | | | | | |
| | | X | | X | | | SKS-MT | | 1 | | | | X | X | | | X | X | X | X | X | X | X | X | X |
| | | X | | X | | | SKS-UM | | 1 | | | X | | | | | | | | | | | | | |

THIS AREA FOR LAB USE ONLY

| | | |
|---|---|---|
| Lab # | Page | of |
| Lab PM | Custody Review | |
| Log In | LIMS Verification | |
| pH | Custody Seals Y N | |
| | Ice Y N | |
| QC Level 1 2 3 Other _____ | | |
| Cooler Temperature | | |

| Alternate Description | Lab ID |
|---|---|
| | |

| | | | |
|---|---|---|---|
| Relinquished By | Empty Bottles | Date/Time | Received By / Empty Bottles / Date/Time |
| Sampled By and Title (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) / Date/Time |
| Received By (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) / Date/Time |
| Received By (Please sign and print name) | | Date/Time | Shipped Via: UPS Fed-Ex Other _____ / Shipping # |

Special Instructions: SAVE SAMPLE FOR 30 DAYS AFTER REPORTING

*Instructions and Agreement Provisions on Reverse Side*

DISTRIBUTION: Original - LAB Yellow - LAB Pink - Client

**Example Chain-of-Custody for JCO Bioassay Sample**

**Send Cannery's copies of Chain-of-Custody forms to:**

**Karen Glatzel, P.O.Box 1238, Trinidad, CA 95570-1238**

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*AND AGREEMENT TO PERFORM SERVICES*

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☐ LRD 5090 Caterpillar Road, Redding, CA 96003-1412, (916) 244-5227 FAX (916) 244-4109
☐ LKW Canviro Analytical Laboratories, Inc., 50 Bathurst, Unit 12, Waterloo, Ontario, Canada N2V 2C5, (519) 747-2575 FAX (519) 747-3806
☐ CVO 2300 NW Walnut Boulevard, Corvallis, OR 97330-3638, (541) 752-4271 FAX (541) 752-0276

COC # _____

| | |
|---|---|
| Project # 147323, JC, BA | Purchase Order # |
| Project Name: JOINT CANNERY BIOASSAY TEST | |
| Company Name: CH2M HILL/GDC | |
| Project Manager or Contact & Phone #: KAREN GLATZEL 707-677-0123, STEVE COSTA | Report Copy to: SAME |
| Requested Completion Date: | Site ID |
| Sample Disposal: Dispose ☒ Return ☐ | |

| Sampling Date | Sampling Time | Type COMP | Type GRAB | Matrix WATER | Matrix SOIL | Matrix AIR | CLIENT SAMPLE ID (9 CHARACTERS) | LAB QC | TOTAL # OF CONTAINERS | Requested Analytical Method # BIOASSAY TEST USING PENAEUS | Preservative NONE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | X | | X | | | SJCO-0200 | | 1 | X | |

DILUTION SERIES:
50, 25, 12.5, 6.25, 3.1
SDS REFERENCE TEST TO BE RUN AS WELL

| THIS AREA FOR LAB USE ONLY | | |
|---|---|---|
| Lab # | Page | of |
| Lab PM | Custody Review | |
| Log In | LIMS Verification | |
| pH | Custody Seals Y N | |
| | Ice Y N | |
| QC Level 1 2 3 Other _____ | | |
| Cooler Temperature | | |
| Alternate Description | Lab ID | |

| | | | |
|---|---|---|---|
| Relinquished By | Empty Bottles | Date/Time | Received By / Empty Bottles / Date/Time |
| Sampled By and Title (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) / Date/Time |
| Received By (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) / Date/Time |
| Received By (Please sign and print name) | | Date/Time | Shipped Via: UPS Fed-Ex Other _____ / Shipping # |

Special Instructions:
MYSIDOPIS BAHIA MAY BE USED IF PENAEUS VANNAMI IS UNAVAILABLE

*Instructions and Agreement Provisions on Reverse Side*

DISTRIBUTION: Original - LAB Yellow - LAB Pink - Client

26 Jan 2000 24 JCO:SOP:REV4

**February 2000 JCO Effluent Sampling Shipping List**

Shipped to:

Mr. Brett Ransby
COS Samoa Packing
Pago Pago, American Samoa 96799

011-684-644-2718 (Office Phone)
011-684-258-1144 (Cell Phone)
011-684

via: Triple B Air Cargo 2/4/00

| Task | Item | Number of Items per Plant | Total Number of Items |
|---|---|---|---|
| **Bioassay Sampling:** | | | |
| Collecting Grab Samples | 1 gallon cubitainers (2 extra in Feb 2000 shipment) | 8 | 16 |
| | 5 gallon cubitainers ( 1 extra in Feb 2000 shipment) | -- | 1 |
| | Coolers to keep samples cold during collection | 2 | 4 |
| Compositing Bioassay Sample: | 1 liter Poly Graduated Cylinder | -- | 1 |
| | Bioassay Compositing Worksheet | 1 | 2 |
| | Bioassay Chain-of-Custody Form | 1 | 2 |
| **Chemistry Sampling:** | | | |
| Folder in Chemistry Supply Cooler | Holding labels, blank worksheets, copies of SOP, blank Chain-of-Custody forms, example return manifests | | 1 |
| Collecting Grab Samples | 1 liter Amber Glass Bottles (24 Total in Feb 2000 shipment) | 8 | 16 |
| | Coolers to keep samples cold during collection | 1 | 2 |
| Chemistry Sample Containers: | Semi-Vols: 1 liter Amber Glass | 1 | 2 |
| | Phenolics: 500 ml poly | 1 | 2 |
| | Metals: 500 ml poly | 2 | 4 |
| Compositing Chemistry Samples: | 1 Gallon Glass Jars | 1 | 2 |
| | 250 ml Glass Graduated Cylinders (pre-cleaned) | 1 | 2 |
| | Chemistry Compositing Worksheet | 1 | 2 |
| | Chemistry Chain-of-Custody Form | 1 | 2 |
| **Sample Preservatives:** | | | |
| Semi-Vol Samples | $H_2SO_4$, 5 ml ampule ( 6 total in Feb 2000 shipment) | 1 | 2 |
| Metals Samples (preserve 1 of 2) | $HNO_3$, 60 ml bottle (use 5 ml in 1 metals sample each in Feb save remainder in a secure location for next sampling) | 1 | 2 |
| Plastic Disposable Pipettes | 5 ml size, use to deliver Nitric Acid to samples | -- | 8 |
| Check pH < 2 in preserved samples | pH paper (1 box to share) | 1 box | 1 box |
| **Other Items:** | | | |
| Nitrile Surgies | | 0.5 box | 1box |
| Marking Pens | | 2 | 4 |
| Calculator | Canneries must supply | -- | -- |
| Strapping Tape | | 2 rolls | 4 rolls |
| Clear Tape | | -- | 1 roll |
| Shipping Labels For Samples | ARI (chemistry), ABT (Bioassay) | -- | 3 each |
| Return Manifests | | | 3 each |
| Ice Chests | | | 3 each |
| Ziplock Baggies | 1 gallon (double bag to hold ice) | | 1 Box |
| Ziplock Baggies | 2 gallon (double bag to hold ice) | | 1 Box |

# ATTACHMENT II

# CHAIN-OF-CUSTODY FORM

**JOINT CANNERY OUTFALL EFFLUENT SAMPLE**
**COS Samoa Packing Company, Inc.**
**StarKist Samoa, Inc.**

**February 2000**

**CH2MHILL Analytical Services**
*CHAIN OF CUSTODY RECORD AND AGREEMENT TO PERFORM SERVICES*

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☐ LCW Canviro Analytical Laboratories, Inc., 50 Bathurst, Unit 12, Waterloo, Ontario, Canada N2V 2C5, (519) 747-2575 FAX (519) 747-3806
☐ CVO 2300 NW Walnut Boulevard, Corvallis, OR 97330-3638, (541) 752-4271 FAX (541) 752-0276

COC # _____

| | |
|---|---|
| Project # 147323.JC.BA | Purchase Order # |
| Project Name: JOINT CANNERY BIOASSAY TEST | |
| Company Name: CH2M HILL/GDC | |
| Project Manager or Contact & Phone #: KAREN GLATZEL 707-677-0123, STEVE COSTA | Report Copy to: SAME |
| Requested Completion Date: | Site ID |
| Sample Disposal: Dispose ☒ Return ☐ | |

| Sampling Date | Sampling Time | Type COMP | Type GRAB | Matrix WATER | Matrix SOIL | Matrix AIR | CLIENT SAMPLE ID (9 CHARACTERS) | LAB QC | TOTAL # OF CONTAINERS | Requested Analytical Method # / Preservative: BIOASSAY TEST USING PENAEUS / NON HG |
|---|---|---|---|---|---|---|---|---|---|---|
| | | X | | X | | | SJCO-0200 | | 1 | X |

DILUTION SERIES:
50, 25, 12.5, 6.25, 3.1
SDS REFERENCE TEST TO BE RUN AS WELL

THIS AREA FOR LAB USE ONLY

| | |
|---|---|
| Lab # | Page ___ of ___ |
| Lab PM | Custody Review |
| Log In | LIMS Verification |
| pH | Custody Seals Y N |
| | Ice Y N |
| QC Level 1 2 3 Other ___ | |
| Cooler Temperature | |

| Alternate Description | Lab ID |
|---|---|
| | |

| | | | |
|---|---|---|---|
| Relinquished By | Empty Bottles | Date/Time | Received By / Empty Bottles / Date/Time |
| X Sampled By and Title: LISINDA S LI Specialist (Please sign and print name) | | Date/Time: 7/9 13:00 | Relinquished By (Please sign and print name) / Date/Time |
| Received By (Please sign and print name) | | Date/Time | Relinquished By (Please sign and print name) / Date/Time |
| Received By (Please sign and print name) | | Date/Time | Shipped Via: UPS Fed-Ex Other ___ / Shipping # |

Special Instructions:
MYSIDOPSIS BAHIA MAY BE USED IF PENAEUS VANNAMI IS UNAVAILABLE

# ATTACHMENT III

## LABORATORY REPORT
## Advanced Biological Testing
## 96-hour Acute Bioassay

**JOINT CANNERY OUTFALL EFFLUENT SAMPLE**
**COS Samoa Packing Company, Inc.**
**StarKist Samoa, Inc.**

**February 2000**

# 1.0
# INTRODUCTION

At the request of CH2M HILL and glatzel da costa (gdc) (Project # 147323.JC.BA), Advanced Biological Testing conducted a four day effluent bioassay test on Mysidopsis bahia using effluents collected from the joint cannery outfall at the StarKist Samoa and COS Samoa Packing tuna canneries in American Samoa. The studies were run using methods generally specified in EPA 1991, per permit numbers AS0000019 and AS0000027 for StarKist and COS Samoa Packing respectively. Penaeus vannami is the preferred species according to the NPDES permit, however when Penaeus are unavailable, EPA has approved Mysidopsis bahia as a substitute. Penaeus was not available to start this test and Mysidopsis was used.

The study was conducted at the Advanced Biological Testing Laboratory in Rohnert Park, California, and was managed by Mr. Mark Fisler.

# 2.0 METHODS

## 2.1 EFFLUENT SAMPLING

The effluents were sampled on February 11, 2000 by cannery personnel under the supervision of CH2M Hill. The laboratory received the sample on February 14, 2000. One five-gallon carboy was provided and maintained in an ice-filled cooler from the date of sampling until laboratory receipt. The sample was at 5°C upon receipt.

## 2.2 SAMPLE PREPARATION

The salinity of the effluent sample was 12.7 ppt and required salinity adjustment to 27 ppt. The effluent salinity was increased to 27 ppt with 100 ppt natural seawater brine. The brine was made from frozen Bodega Bay seawater. Due to the dilution of the effluent with the brine solution, the initial maximum concentration of effluent was 83.6%. The highest initial test concentration was made by diluting the 80% effluent with Bodega Bay seawater to an actual effluent concentration of 50%. The dissolved oxygen level in the sample was low. The initial total ammonia was approximately 6.8 ppm (3.37 ppm in the 50% test sample).

The effluents were tested at an actual effluent concentration series of 50%, 25%, 12.5%, 6.25%, and 3.1% as vol:vol dilution in seawater. A brine control was run with the test to assess the potential toxicity from the added brine. The diluent and the control water were filtered seawater from Bodega Bay. The dilutions were brought to the test temperature (20 ± 2°C) and aerated continuously. Based upon the previous testing, these effluents have an increasing biological oxygen demand, with a significant peak at 10-14 hours after test initiation. Previous testing of this effluent conducted without initial aeration demonstrated significant toxicity at 24 hours (or before); therefore aeration was carried out from the beginning of the test. According to EPA methods the test chambers were renewed with retained effluents held under refrigeration from test initiation on Day 2.

A reference toxicant was run using concentrations initially provided by the EPA. The toxicant was sodium dodecyl sulfonate (SDS) made up as a 2 grams per liter stock solution in distilled water. The tested concentrations were set at 50, 25, 12.5, 6.25, 3.1 mg/L in 27 ppt seawater.

## 2.3 TESTING PROCEDURES

The bioassays were carried out on two day old larvae of Mysidopsis bahia supplied by Aquatox in Arkansas. The mysids were received on February 15, 2000 and were used immediately. Five replicates of each concentration were tested with ten animals per replicate. Water quality was monitored daily as initial quality on Day 0 and final water quality on Days 1-4. Parameters measured included dissolved oxygen, pH, salinity, total ammonia, and temperature.

## 2.4 STATISTICAL ANALYSIS

At the conclusion of the test, the survival data were evaluated statistically using ToxCalc™ to determine ECp, NOEC, and LOEC values where appropriate. ToxCalc™ is a comprehensive statistical application that follows standard guidelines for acute toxicity data analysis. Statistical effects can be measured by the ECp, the estimated concentration that causes any effect, either lethal (LC) or sublethal (IC), on p% of the test population. The LCp is the point estimate of the concentration at which a lethal effect is observed in p% of the test organisms. ECp values include 95% confidence limits if calculable.

# 3.0
# RESULTS

## 3.1 INTRODUCTION

Tables 1 through 6 present the results of the Mysidopsis testing. The test conditions are summarized in Table 1. In the test, water quality measurements were within the acceptable limits provided in EPA 1991. Temperature was maintained at 20 ± 2°C; the pH remained relatively stable, and the salinity increased very slightly as would be expected in a static test (Tables 2 and 3). Aeration was maintained in all chambers for the duration of the test. The test solutions were renewed with reserved effluent at 48 hrs.

Initial ammonia was 3.37 ppm in the 50% effluent and was proportionally diluted at lower percentage concentrations. The LC50 for the effluent was 19.95% (95% confidence limits = 14% to 22%). There was significant mortality at the 12.5%, 25% and 50% concentrations compared to the control (Table 4). The NOEC was 6.25%, and the LOEC was 12.5%. The TU was 16.

The reference toxicant test had an LC50 of 15.4 mg/L (Tables 5 and 6). The laboratory mean for *Mysidopsis bahia* was 14.67 mg/L ± 8.21mg/L. The data is within one standard deviation of the laboratory mean, indicating normal sensitivity.

**Advanced Biological Testing Inc.**

**TABLE 1**

**Bioassay Procedure And Organism Data**

**For the Survival Bioassay**

**Using Mysidopsis bahia(U.S. EPA 1991)**

| Parameter | Data |
|---|---|
| **Sample Identification** | |
| Sample ID(s) | 000214-1 |
| Date Sampled | 2/11/00 |
| Date Received at ABT | 2/14/00 |
| Volume Received | Five gallons |
| Sample Storage Conditions | 4°C in the dark |
| **Test Species** | Mysidopsis bahia |
| Supplier | Aquatox, Hot Springs, Arkansas |
| Collection location | In house colony |
| Date Acquired | 2/15/00 |
| Acclimation Time | Used immediately |
| Acclimation Water | Shipping water |
| Acclimation Temperature | 20±2°C |
| Age group | Two day old larvae |
| **Test Procedures** | |
| Type; Duration | Acute, static/renewal at 48 hours |
| Test Dates | 2/15/00-2/19/00 |
| Control Water | Bodega Bay seawater |
| Test Temperature | 20± 2°C |
| Test Photoperiod | 14 L : 10 D |
| Salinity | 27± 2 ppt |
| Test Chamber | 1000 mL jars |
| Animals/Replicate | 10 |
| Exposure Volume | 500 mL |
| Replicates/Treatment | 5 |
| Feeding | Brine shrimp (<24 hr old nauplii) |
| Deviations from procedures | None |

**Table 2**

**INITIAL WATER QUALITY MEASUREMENTS**
**For Acute Mysid Effluent Test**
**Test Dates: 2/15/00 - 2/19/00**

| Concentration | Day 0 | | | | | Day 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (%) | pH | DO | NH 3 | °C | Sal | pH | DO | NH 3 | °C | Sal |
| **Control** | 8.23 | 7.9 | 0.03 | 18.5 | 27 | 8.00 | 8.1 | 0.03 | 19.7 | 27 |
| **Brine** | 8.14 | 7.8 | | 18.3 | 27 | 8.02 | 7.8 | | 18.5 | 27 |
| **3.1** | 8.12 | 7.9 | | 19.3 | 27 | 7.82 | 6.7 | | 19.0 | 27 |
| **6.25** | 8.02 | 7.9 | | 19.3 | 27 | 7.70 | 6.1 | | 19.1 | 27 |
| **12.5** | 7.77 | 7.7 | | 19.1 | 27 | 7.49 | 5.8 | | 19.7 | 27 |
| **25** | 7.50 | 6.5 | | 18.7 | 27 | 7.34 | 5.2 | | 19.8 | 27 |
| **50** | 7.08 | 4.9 | 3.37 | 18.3 | 27 | 7.20 | 5.3 | 3.29 | 19.9 | 27 |
| **Min** | 7.08 | 4.9 | 0.03 | 18.3 | 27 | 7.20 | 5.2 | 0.03 | 18.5 | 27 |
| **Max** | 8.23 | 7.9 | 3.37 | 19.3 | 27 | 8.02 | 8.1 | 3.29 | 19.9 | 27 |

**Table 3**

**Final Water Quality Measurements For the Acute Mysid Effluent Test**

| Concentration | | Day 1 | | | | Day 2 | | | | Day 3 | | | | Day 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (%) | Rep | pH | DO | °C | Sal | pH | DO | °C | Sal | pH | DO | °C | Sal | pH | DO | °C | Sal |
| **Control** | 1 | 8.15 | 7.2 | 19.7 | 27 | 8.18 | 7.9 | 19.3 | 28 | 8.00 | 7.5 | 19.0 | 28 | 7.98 | 7.8 | 19.1 | 29 |
| | 2 | 8.14 | 7.3 | 19.7 | 27 | 8.20 | 7.8 | 19.3 | 28 | 8.01 | 7.5 | 19.0 | 28 | 7.98 | 7.7 | 19.2 | 29 |
| | 3 | 8.14 | 7.3 | 19.7 | 28 | 8.20 | 7.8 | 19.3 | 28 | 8.01 | 7.5 | 19.1 | 28 | 8.00 | 7.6 | 19.2 | 29 |
| | 4 | 8.14 | 7.4 | 19.7 | 27 | 8.21 | 7.7 | 19.3 | 28 | 8.02 | 7.5 | 19.1 | 28 | 8.02 | 7.6 | 19.3 | 29 |
| | 5 | 8.14 | 7.5 | 19.7 | 27 | 8.18 | 7.7 | 19.3 | 28 | 8.04 | 7.5 | 19.1 | 28 | 8.02 | 7.6 | 19.2 | 29 |
| **Brine** | 1 | 8.12 | 7.6 | 19.8 | 27 | 8.16 | 7.6 | 19.4 | 28 | 8.02 | 7.5 | 19.0 | 28 | 8.00 | 7.5 | 19.2 | 29 |
| | 2 | 8.12 | 7.5 | 19.8 | 27 | 8.14 | 7.6 | 19.4 | 28 | 8.02 | 7.5 | 19.1 | 28 | 8.02 | 7.5 | 19.3 | 29 |
| | 3 | 8.12 | 7.5 | 19.8 | 27 | 8.14 | 7.6 | 19.4 | 28 | 8.05 | 7.5 | 19.1 | 28 | 8.05 | 7.5 | 19.2 | 29 |
| | 4 | 8.14 | 7.5 | 19.8 | 27 | 8.17 | 7.5 | 19.4 | 28 | 8.06 | 7.6 | 19.1 | 28 | 8.06 | 7.4 | 19.3 | 29 |
| | 5 | 8.14 | 7.5 | 19.8 | 27 | 8.16 | 7.5 | 19.4 | 28 | 8.08 | 7.5 | 19.1 | 28 | 8.06 | 7.4 | 19.3 | 29 |
| **3.1** | 1 | 8.14 | 7.6 | 19.8 | 27 | 8.10 | 7.5 | 19.4 | 28 | 8.04 | 7.5 | 19.1 | 28 | 8.05 | 7.4 | 19.3 | 29 |
| | 2 | 8.08 | 7.5 | 19.8 | 27 | 8.06 | 7.4 | 19.4 | 28 | 8.06 | 7.5 | 19.1 | 28 | 8.04 | 7.4 | 19.4 | 29 |
| | 3 | 8.09 | 7.5 | 19.8 | 27 | 8.10 | 7.3 | 19.4 | 28 | 8.04 | 7.5 | 19.2 | 28 | 8.02 | 7.4 | 19.3 | 29 |
| | 4 | 8.06 | 7.5 | 19.8 | 27 | 8.09 | 7.3 | 19.4 | 28 | 8.04 | 7.5 | 19.2 | 28 | 8.02 | 7.4 | 19.4 | 29 |
| | 5 | 8.08 | 7.5 | 19.8 | 27 | 8.13 | 7.3 | 19.4 | 28 | 8.02 | 7.5 | 19.2 | 28 | 8.04 | 7.4 | 19.3 | 29 |
| **6.25** | 1 | 8.12 | 7.5 | 19.8 | 27 | 8.18 | 7.3 | 19.4 | 28 | 8.05 | 7.5 | 19.2 | 28 | 8.06 | 7.4 | 19.4 | 29 |
| | 2 | 8.14 | 7.5 | 19.9 | 27 | 8.20 | 7.3 | 19.5 | 28 | 8.06 | 7.5 | 19.3 | 28 | 8.06 | 7.4 | 19.4 | 29 |
| | 3 | 8.13 | 7.5 | 19.9 | 27 | 8.21 | 7.3 | 19.5 | 28 | 7.99 | 7.5 | 19.2 | 28 | 8.06 | 7.3 | 19.4 | 29 |
| | 4 | 8.02 | 7.5 | 19.8 | 27 | 8.09 | 7.3 | 19.4 | 28 | 7.95 | 7.5 | 19.2 | 28 | 7.98 | 7.2 | 19.4 | 29 |
| | 5 | 8.04 | 7.4 | 19.8 | 27 | 8.06 | 7.2 | 19.4 | 28 | 7.93 | 7.4 | 19.1 | 28 | 7.99 | 7.2 | 19.3 | 29 |
| **12.5** | 1 | 8.02 | 7.4 | 19.8 | 27 | 8.08 | 7.3 | 19.5 | 28 | 7.94 | 7.3 | 19.3 | 28 | 8.01 | 7.2 | 19.5 | 29 |
| | 2 | 8.02 | 7.5 | 19.8 | 27 | 8.08 | 7.2 | 19.5 | 28 | 7.97 | 7.3 | 19.3 | 28 | 8.02 | 7.2 | 19.5 | 29 |
| | 3 | 8.04 | 7.4 | 19.9 | 27 | 8.09 | 7.1 | 19.5 | 28 | 7.96 | 7.3 | 19.3 | 28 | 8.02 | 7.2 | 19.5 | 29 |
| | 4 | 8.04 | 7.4 | 19.8 | 27 | 8.08 | 7.1 | 19.5 | 28 | 7.97 | 7.2 | 19.2 | 28 | 8.02 | 7.1 | 19.4 | 29 |
| | 5 | 8.01 | 7.4 | 19.8 | 27 | 8.06 | 7.1 | 19.4 | 28 | 7.93 | 7.2 | 19.2 | 28 | 7.99 | 7.0 | 19.3 | 29 |
| **25** | 1 | 8.06 | 7.4 | 19.8 | 28 | 8.18 | 7.2 | 19.4 | 28 | 7.92 | 7.2 | 19.2 | 28 | 8.06 | 7.0 | 19.4 | 29 |
| | 2 | 8.08 | 7.4 | 19.9 | 27 | 8.21 | 7.2 | 19.5 | 28 | 7.99 | 7.1 | 19.3 | 28 | 8.13 | 7.0 | 19.5 | 29 |
| | 3 | 8.02 | 7.4 | 19.9 | 27 | 8.08 | 7.1 | 19.5 | 28 | — | — | — | — | — | — | — | — |
| | 4 | 8.00 | 7.3 | 19.8 | 27 | 8.06 | 7.1 | 19.5 | 28 | 8.08 | 7.1 | 19.3 | 28 | 8.14 | 7.0 | 19.4 | 29 |
| | 5 | 8.02 | 7.3 | 19.8 | 27 | 8.05 | 7.1 | 19.4 | 28 | 8.08 | 7.2 | 19.3 | 28 | 8.16 | 7.0 | 19.3 | 29 |
| **50** | 1 | 8.04 | 7.3 | 19.7 | 27 | — | — | — | — | — | — | — | — | — | — | — | — |
| | 2 | 8.02 | 7.3 | 19.6 | 27 | — | — | — | — | — | — | — | — | — | — | — | — |
| | 3 | 8.06 | 7.3 | 19.8 | 28 | 8.30 | 7.1 | 19.3 | 28 | 8.17 | 7.3 | 19.0 | 28 | 8.26 | 7.0 | 19.3 | 29 |
| | 4 | 8.14 | 7.0 | 19.8 | 27 | | | | | | | | | | | | |
| | 5 | 8.05 | 6.9 | 19.7 | 27 | | | | | | | | | | | | |
| **Min** | | 8.00 | 6.9 | 19.6 | 27 | 8.05 | 7.1 | 19.3 | 28 | 7.92 | 7.1 | 19.0 | 28 | 7.98 | 7.0 | 19.1 | 29 |
| **Max** | | 8.15 | 7.6 | 19.9 | 28 | 8.30 | 7.9 | 19.5 | 28 | 8.17 | 7.6 | 19.3 | 28 | 8.26 | 7.8 | 19.5 | 29 |

Note: — = All animals dead.

Table 4

**Summary of Results for the Mysid Acute Effluent Test**

| Concentration (%) | Rep | Initial Added | Day 1 | Day 2 | Day 3 | Day 4 | % Survival | Average % Survival |
|---|---|---|---|---|---|---|---|---|
| **Control** | 1 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 2 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 3 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 4 | 10 | 10 | 10 | 10 | 9 | 90 | |
| | 5 | 10 | 10 | 10 | 10 | 10 | 100 | 98.0 |
| **Brine** | 1 | 10 | 10 | 10 | 10 | 9 | 90 | |
| | 2 | 10 | 10 | 10 | 9 | 9 | 90 | |
| | 3 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 4 | 10 | 10 | 10 | 9 | 9 | 90 | |
| | 5 | 10 | 10 | 10 | 10 | 10 | 100 | 94.0 |
| **3.1** | 1 | 10 | 10 | 9 | 9 | 8 | 80 | |
| | 2 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 3 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 4 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 5 | 10 | 10 | 10 | 10 | 10 | 100 | 96.0 |
| **6.25** | 1 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 2 | 10 | 10 | 10 | 10 | 10 | 100 | |
| | 3 | 10 | 9 | 9 | 9 | 9 | 90 | |
| | 4 | 10 | 10 | 10 | 10 | 9 | 90 | |
| | 5 | 10 | 10 | 10 | 10 | 10 | 100 | 96.0 |
| **12.5** | 1 | 10 | 10 | 9 | 8 | 7 | 70 | |
| | 2 | 10 | 10 | 10 | 9 | 7 | 70 | |
| | 3 | 10 | 10 | 10 | 9 | 8 | 80 | |
| | 4 | 10 | 9 | 9 | 8 | 8 | 80 | |
| | 5 | 10 | 10 | 10 | 10 | 9 | 90 | 78.0 |
| **25** | 1 | 10 | * | 10 | 3 | 0 | 0 | |
| | 2 | 10 | * | 10 | 8 | 7 | 70 | |
| | 3 | 10 | * | 0 | — | — | 0 | |
| | 4 | 10 | * | 9 | 8 | 6 | 60 | |
| | 5 | 10 | * | 10 | 5 | 5 | 50 | 36.0 |
| **50** | 1 | 10 | 0 | — | — | — | 0 | |
| | 2 | 10 | 0 | — | — | — | 0 | |
| | 3 | 10 | 10 | 5 | 1 | 0 | 0 | |
| | 4 | 10 | 0 | — | — | — | 0 | |
| | 5 | 10 | 0 | — | — | — | 0 | 0.0 |

Note: — = All animals dead.
* = Water too turbid to count.

**Table 5**

**Water Quality Measurements for the Mysid Reference Toxicant Bioassay**

| Concentration | | Day 0 | | | | Day 1 | | | | Day 2 | | | | Day 3 | | | | Day 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (mg/L) | Rep | pH | DO | °C | Sal | pH | DO | °C | Sal | pH | DO | °C | Sal | pH | DO | °C | Sal | pH | DO | °C | Sal |
| **Control** | **1** | 8.05 | 7.9 | 18.6 | 27 | 8.08 | 6.7 | 19.5 | 27 | 7.93 | 6.6 | 18.8 | 28 | 7.92 | 6.4 | 18.8 | 28 | 7.87 | 6.4 | 19.2 | 29 |
| | **2** | | | | | 8.08 | 6.7 | 19.5 | 27 | 7.94 | 6.5 | 18.9 | 28 | 7.91 | 6.3 | 18.8 | 28 | 7.88 | 6.4 | 19.3 | 29 |
| | **3** | | | | | 8.09 | 6.7 | 19.7 | 27 | 7.93 | 6.5 | 19.1 | 28 | 7.89 | 6.1 | 19.0 | 28 | 7.89 | 6.4 | 19.4 | 29 |
| **3.1** | **1** | 8.08 | 7.9 | 18.8 | 27 | 8.09 | 6.7 | 19.7 | 27 | 7.94 | 6.5 | 19.2 | 28 | 7.88 | 6.0 | 19.1 | 28 | 7.87 | 6.3 | 19.4 | 29 |
| | **2** | | | | | 8.04 | 6.7 | 19.8 | 27 | 7.93 | 6.4 | 19.2 | 28 | 7.87 | 6.0 | 19.1 | 28 | 7.86 | 6.3 | 19.5 | 29 |
| | **3** | | | | | 8.02 | 6.6 | 19.8 | 27 | 7.91 | 6.4 | 19.3 | 28 | 7.87 | 6.0 | 19.1 | 28 | 7.86 | 6.2 | 19.5 | 29 |
| **6.25** | **1** | 8.10 | 7.9 | 18.9 | 27 | 7.99 | 5.3 | 19.8 | 27 | 7.90 | 6.3 | 19.3 | 28 | 7.86 | 5.9 | 19.2 | 28 | 7.86 | 6.2 | 19.5 | 29 |
| | **2** | | | | | 7.95 | 5.3 | 19.8 | 27 | 7.87 | 6.3 | 19.4 | 28 | 7.85 | 5.9 | 19.2 | 28 | 7.85 | 6.2 | 19.5 | 29 |
| | **3** | | | | | 7.93 | 5.3 | 19.8 | 27 | 7.83 | 6.3 | 19.4 | 28 | 7.83 | 5.8 | 19.2 | 28 | 7.84 | 6.1 | 19.5 | 29 |
| **12.5** | **1** | 8.10 | 7.8 | 19.0 | 27 | 7.91 | 5.3 | 19.8 | 27 | 7.83 | 5.4 | 19.4 | 28 | 7.82 | 5.7 | 19.2 | 28 | 7.83 | 6.0 | 19.4 | 29 |
| | **2** | | | | | 7.96 | 5.4 | 19.8 | 27 | 7.79 | 5.1 | 19.2 | 28 | 7.81 | 5.7 | 19.2 | 28 | 7.81 | 6.0 | 19.4 | 29 |
| | **3** | | | | | 7.98 | 5.4 | 19.8 | 27 | 7.78 | 5.1 | 19.3 | 28 | 7.80 | 5.7 | 19.1 | 28 | 7.79 | 6.0 | 19.4 | 29 |
| **25** | **1** | 8.13 | 7.8 | 19.0 | 27 | 7.99 | 5.6 | 19.8 | 27 | 7.77 | 5.1 | 19.2 | 27 | — | — | — | — | — | — | — | — |
| | **2** | | | | | 8.02 | 5.7 | 19.8 | 27 | 7.78 | 5.2 | 19.2 | 27 | — | — | — | — | — | — | — | — |
| | **3** | | | | | 8.08 | 5.9 | 19.8 | 27 | 7.79 | 5.3 | 19.2 | 27 | — | — | — | — | — | — | — | — |
| **50** | **1** | 8.14 | 7.8 | 19.1 | 26 | 8.06 | 6.0 | 19.5 | 26 | — | — | — | — | — | — | — | — | — | — | — | — |
| | **2** | | | | | 8.08 | 6.1 | 19.5 | 26 | — | — | — | — | — | — | — | — | — | — | — | — |
| | **3** | | | | | 8.09 | 6.1 | 19.4 | 26 | — | — | — | — | — | — | — | — | — | — | — | — |
| **Min** | | 8.05 | 7.8 | 18.6 | 26 | 7.91 | 5.3 | 19.4 | 26 | 7.77 | 5.1 | 18.8 | 27 | 7.80 | 5.7 | 18.8 | 28 | 7.79 | 6.0 | 19.2 | 29 |
| **Max** | | 8.14 | 7.9 | 19.1 | 27 | 8.09 | 6.7 | 19.8 | 27 | 7.94 | 6.6 | 19.4 | 28 | 7.92 | 6.4 | 19.2 | 28 | 7.89 | 6.4 | 19.5 | 29 |

Note: — = All animals dead.

**Table 6**

**Summary of Results for the Mysid Reference Toxicant Bioassay**

| Concentration (mg/L) | Rep | Initial Added | Day 1 | Day 2 | Day 3 | Day 4 | % Survival | Average % Survival |
|---|---|---|---|---|---|---|---|---|
| **Control** | **1** | 10 | 10 | 10 | 10 | 10 | 100 | |
| | **2** | 10 | 10 | 10 | 10 | 10 | 100 | |
| | **3** | 10 | 10 | 9 | 8 | 8 | 80 | 93.3 |
| **3.1** | **1** | 10 | 9 | 9 | 9 | 9 | 90 | |
| | **2** | 10 | 10 | 10 | 10 | 9 | 90 | |
| | **3** | 10 | 10 | 10 | 9 | 9 | 90 | 90.0 |
| **6.25** | **1** | 10 | 10 | 10 | 9 | 9 | 90 | |
| | **2** | 10 | 10 | 10 | 10 | 9 | 90 | |
| | **3** | 10 | 10 | 10 | 10 | 9 | 90 | 90.0 |
| **12.5** | **1** | 10 | 10 | 10 | 9 | 8 | 80 | |
| | **2** | 10 | 10 | 10 | 8 | 6 | 60 | |
| | **3** | 10 | 10 | 10 | 9 | 7 | 70 | 70.0 |
| **25** | **1** | 10 | 9 | 0 | — | — | 0 | |
| | **2** | 10 | 9 | 0 | — | — | 0 | |
| | **3** | 10 | 8 | 0 | — | — | 0 | 0.0 |
| **50** | **1** | 10 | 0 | — | — | — | 0 | |
| | **2** | 10 | 0 | — | — | — | 0 | |
| | **3** | 10 | 0 | — | — | — | 0 | 0.0 |

LC50 = 15.4 mg/L.
Laboratory mean = 14.67 ± 8.21.
Acceptable sensitivity.

# 4.0
# REFERENCES

U.S. EPA. 1993. Methods for measuring acute toxicity of effluents to freshwater and marine organisms, 4th ed. EPA 600/4-90/027F, August, 1993.